SEPARATION OF BIO-POLYESTER FROM BIO-POLYESTER-CONTAINING MICROORGANISM

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Abstract of **JP7031489**

PURPOSE:To provide a method for efficiently separating a bio-polyester in a granular state from microbial cells containing the bio-polyester. CONSTITUTION:This method for separating the granular bio-polyester comprises adding an alkali in an amount of 1mmol-1mol/kg microbial cells to the aqueous suspension of bio-polyester-containing microorganisms, charging the suspension into a pressure-resistant container or preliminarily heating the suspension at 40-100 deg.C and then charging the heated suspension into the pressure-resistant container, and subsequently heating and retaining the charged suspension at 40-100 deg.C for raising the pressure to spout the suspension from the small opening of the container, thus allowing the shearing force of the fluid to act on the microorganism.

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(54)【発明の名称】　バイオポリエステル含有微生物からのバイオポリエステルの分離方法

(57)【要約】

【目的】　バイオポリエステル含有微生物から、バイオポリエステルを効率よく顆粒状で分離する方法を提供する。

【構成】　バイオポリエステル含有微生物の水性懸濁液に１mmol／kg菌体～１mol　／kg菌体の量のアルカリを添加した後、該懸濁液を耐圧性容器に導入し、もしくは予め該懸濁液を４０～１００℃に加熱して耐圧性容器に導入し、４０～１００℃に加熱，保温して高圧をかけ、該容器の微小開口部から懸濁液を噴出させることにより微生物に流体剪断力を作用させ、顆粒状のバイオポリエステルを分離する。

latus等、シュウドモナス属（Pseudomonas）、バシルス属（Bacillus）、アゾトバクター属（Azotobacter）、ノカルディア属（Nocardia）等の菌株が示されるが、その種類に限定されるものではない。ここで、バイオポリエステルとは、ポリ－D－3－ヒドロキシブチレート〔以下、P（3HB）と略称する〕をはじめとするポリヒドロキシアルカノエート〔以下、P（HA）と略称する〕と称される微生物産生ポリエステルを指す。P（3HB）以外の代表的な例として、3HBとD－3－ヒドロキシバレレート（3HV）との共重合体〔P. A. Holmes et al（ICI）, Eur. Pat. App l, 0052459（1981）〕、3HBと4－ヒドロキシブチレート（4HB）との共重合体〔Y. Doi et al., Macromolecules, 21, 2722（1988）〕が挙げられる。細胞内に蓄積しているバイオポリエステルは、微小な顆粒として存在することが知られている。

【0007】処理される細胞内のバイオポリエステル含有率（以下、ポリマー含有率という）は、高いほうが好ましい。一般に、乾燥菌体としてポリマー含有率が20重量%以上がよい。アルカリ添加量、処理時間、分離操作の効率、分離ポリマーの純度等を考慮すると、50重量%以上のポリマー含有率が特に好ましい。水性懸濁液とは、培養終了後の培養懸濁液そのもの、または培養液から遠心等で分離した菌体を水に懸濁させたものを指す。菌体の懸濁濃度は、乾燥菌体換算で150g／l以下、好ましくは100g／l以下である。使用するアルカリとしては、NaOHを始めとしてLiOH、KOH等を含めたアルカリ金属の水酸化物、あるいはNH4OHが用いられる。アルカリの使用量は1mmol／kg菌体～1mol／kg菌体、好ましくは2．5mmol／kg菌体～200mmol／kg菌体、特に好ましくは50mmol／kg菌体～200mmol／kg菌体で、これを微生物の水性懸濁液に添加する。

【0008】本発明の方法では、アルカリを添加後は、水性懸濁液は、微小開口部を有する耐圧性容器に導入され、高圧をかけられる。このようにして開口部から押し出される菌体には、大きな剪断力が働くため、菌体は破壊され、バイオポリエステルの分離が促進されると推定される。このような耐圧性容器と加圧機構からなる装置は、循環装置付高圧ホモジナイザーによって代表される。したがって、本発明のバイオポリエステル分離法は、高圧ホモジナイザーの利用によって実施可能となる。高圧ホモジナイザーの温度設定は40～100℃、好ましくは60～100℃にする。該懸濁液の加熱は、高圧ホモジナイザーの導入前に、設定温度に加熱しておくことも望ましい。高圧ホモジナイザー内に導入した該懸濁液にかける圧力は、装置によるが、500～1500kgf／cm² で作用させるのが好ましい。

【0009】循環装置付高圧ホモジナイザーとしては、マントンゴーリン（独国APV・ゴーリン社製）、ミニラボ（デンマーク　APVラニー社製）、ブランリューベ連続式細胞破砕機（独国Bran+Luebbe社製）、マイクロフルイダイザー（米国Microfluidics社製）等を用いることができる。これらの装置は、一般的に液体を加圧することによって、乳化・分散・細胞破砕等に用いられることがよく知られている。本発明では、高圧ホモジナイザー内での加熱が必須なので、類似の高圧ホモジナイザーの一種であるが非加熱型であるフレンチプレスは、本発明に不適当である。フレンチプレスを用いて微生物中のバイオポリエステルを分離することは知られているが（Helmut Brandl etal., Advances in Biochemical Engineering, Biotechnology（1990）, 41, 77－93.）、本発明の技術的特徴であるアルカリ添加や、加熱による分離の協同効果を実現した例は知られていない。

【0010】以上の処理操作により、短時間で効率よく菌体壁を破壊し、バイオポリエステルを顆粒状で菌体から分離できる。菌体壁が破壊されると、核酸のような水溶性の高分子物質が細胞外に溶出するために、該懸濁液の粘度は一旦上昇するが、剪断力によって核酸分子の切断も起こるためか、該懸濁液の粘度が再び低下し、その後の遠心操作、ろ過操作等でのバイオポリエステルの分離が容易に行える。処理前の該懸濁液の菌体濃度は、乾燥菌体換算で150g菌体／lまで処理可能であるため、通常培養後の菌体濃度を薄める必要がない。本発明により、短時間で効率良く菌体壁が破壊され、バイオポリエステルを顆粒状で分離できる。

【0011】

【実施例】本実施例で用いた微生物は、アルカリゲネス属に属する微生物アルカリゲネス・リポリティカ（Alcaligenes lipolytica）AK201（特開平5－64592）で、培養後、P（3HB）を約50wt%含有している菌を遠心（8000rpm, 10min, 遠心分離機はKUBOTA製6810使用）によって培養液から分離後、ペースト状菌体に水を加えて40g菌体／lの水性懸濁液とした。この水性懸濁液を用いて、以下に示す実施例1、2および比較例1～4を行った。

【0012】実施例1、2および比較例1～4の操作で得たP（3HB）は、純度を調べるためにガスクロマトグラフィー、分子量分布の決定にゲルパーミエーションクロマトグラフィー（GPC）を用いて分析を行った。なお、ガスクロマトグラフィーには、実施例1、2および比較例1～4で得られた沈澱物を乾燥（105℃, 24hr）した後、メタノール／硫酸（85／15　wt%／wt%）によりメタノリシスして菌体内ポリエステ

PATENT ABSTRACTS OF JAPAN

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(54) SEPARATION OF BIO-POLYESTER FROM BIO-POLYESTER-CONTAINING MICROORGANISM

(57)Abstract:
PURPOSE: To provide a method for efficiently separating a bio-polyester in a granular state from microbial cells containing the bio-polyester.
CONSTITUTION: This method for separating the granular bio-polyester comprises adding an alkali in an amount of 1mmol-1mol/kg microbial cells to the aqueous suspension of bio-polyester-containing microorganisms, charging the suspension into a pressure-resistant container or preliminarily heating the suspension at 40-100° C and then charging the heated suspension into the pressure-resistant container, and subsequently heating and retaining the charged suspension at 40-100° C for raising the pressure to spout the suspension from the small opening of the container, thus allowing the shearing force of the fluid to act on the microorganism.

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CLAIMS

[Claim(s)]
[Claim 1] It is 1 mmol/kg biomass -1mol to the aqueous suspension of a biotechnology polyester content microorganism. After adding the alkali of the amount of a /kg biomass, Introduce this suspension into a pressure-resistant container, and it is heated and kept warm within the limits of 40-100 degrees C. The separation approach of the biotechnology polyester from the biotechnology polyester content microorganism characterized by applying high voltage to this suspension, making liquid shearing force act on a microorganism by gushing this suspension from minute opening of this container, and separating granularity biotechnology polyester.
[Claim 2] The method according to claim 1 of heating aqueous suspension in the range of 40-100 degrees C beforehand, before introducing into a pressure-resistant container.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the separation approach of having biodegradability, from the biomass of biotechnology polyester.
[0002]
[Description of the Prior Art] Although current and a plastic waste are processed by incineration, reclamation, etc., there are problems, such as warming of the earth and ground relaxation of a reclaimed ground, in these arts, respectively. Therefore, recycle system-ization is progressing with a rise of the social consciousness to plastics recycle. However, there is much what remains there being a limitation in a recyclable application, could not respond only by incineration, reclamation, and recycle as a plastic waste art as a practical question, and left in natural environment. Then, after abolition, it is incorporated by the cyclical change of materials of a nature, a biodegradable plastic from which a decomposition product does not serve as harmful matter attracts attention, and the development is furthered. As such plastics, especially the polyester that a microorganism generates within a biomass is expected that it is included in the carbon cycle process of a nature, and stabilization of an ecosystem is made. Moreover, also in the medical field, the implant material of recovery needlessness and the utilization as a drug carrier are possible.
[0003] However, in order to use this polyester as plastics, it is necessary to dissociate and to take out from the inside of the biomass of a microorganism, As an approach of obtaining biotechnology polyester from a biotechnology polyester content microorganism, a biomass is dissolved using the extraction method by organic solvents including chloroform, sodium hypochlorite (Williamson, D.H., and Wilkinson, J.F. (1958), J.Gen.Microbiol.19,198-203.), or a lysozyme, and the method of collecting the polymers which remained as granulation is learned. In addition, a biomass is destroyed by disconnection of the pressure of the high-pressure steam of the approach (JP,60-145097,A) and 100-degree-C ** which collect polymers by the dissolution of the biomass by specific enzymes other than a lysozyme etc., and there is the approach (JP,57-174094,A) of dividing into biomass fragment waste and a polymer etc.
[0004]
[Problem(s) to be Solved by the Invention] However, the solvent extraction method by chloroform etc. needs not only the extracting solvent concerned but the poor solvent for reprecipitation for a large quantity. Therefore, if it is going to reuse a solvent respectively, it is required to separate two sorts of solvents. Furthermore, since it is required to dry the whole biomass thoroughly in advance of solvent extraction generally and it also becomes requiring great heat energy, in order to produce biotechnology polyester industrially, much the facility for processes and energy are needed, and it is disadvantageous as a matter of fact. Although the fault of a solvent extraction method can be avoided when it processes by sodium hypochlorite, on the other hand, molecular weight lowering of polyester takes place (J. A.Ramsay, E.Berger, B.A.Ramsay and C.Chavarie (1990), J.Biotechnology Techniques 4, 4,221-226), and a problem arises in the quality of a polymer. Although it is effective for little experimental utilization, since an enzyme like a lysozyme is difficult to secure a large quantity, it is not suitable for the mass

production of biotechnology polyester. In the enzymatic process of JP,60-145097,A, the actuation before and behind enzyme processing becomes a multistage story, and, in addition, the room of an improvement is large for mass production. Since the approach by release of the pressure of JP,57-174094,A has not indicated the purity or yield of polyester which were obtained, its effectiveness is unknown. This invention aims at offering the approach of separating biotechnology polyester from the microorganism containing biotechnology polyester by applying shearing force at less than 100 degrees C in an aquosity medium without using an organic solvent.
[0005]
[Means for Solving the Problem] This invention is 1 mmol/kg biomass -1mol to the aqueous suspension of a biotechnology polyester content microorganism. /biomass, Preferably A 2.5 mmol/kg biomass - 200 mmol/kg biomass. The alkali of the amount of a 50mmol(s) - 200 mmol/kg biomass is added especially preferably. Introduce this suspension into a pressure-resistant container, or heat this suspension in the range of 40-100 degrees C beforehand, and it introduces into a pressure-resistant container. It is heated and kept warm within the limits of 40-100 degrees C, and fluid shearing force is made to act on a microorganism by gushing this suspension, and it is related with the separation approach of the biotechnology polyester from the biotechnology polyester content microorganism characterized by separating granularity biotechnology polyester. In addition, it is the approach this invention promotes separation of biomass destruction and biotechnology polyester to this destroying a biomass by the pressure shock at the time of low-voltage-izing high voltage rapidly although high voltage is similarly used by the approach of JP,57-174094,A according to the shearing force at the time of minute opening injection of a high voltage liquid.
[0006] The microorganisms used for this invention are bacteria (bacteria) which are accumulating biotechnology polyester in intracellular. For example, the bacillus of Alcaligenes (Alcaligenes), A.lipolytica Although strain, such as Pseudomonas (Pseudomonas), such as AK201 (JP,5-64592,A), A.eutrophus, and A.latus, a bacillus group (Bacillus), an azotobacter group (Azotobacter), and a Nocardia group (Nocardia), is shown, it is not limited to the class. Here, biotechnology polyester points out the microorganism production polyester called polyhydroxy alkanoate [it is hereafter called P (HA) for short] including Poly D-3-hydroxy butyrate [it is hereafter called P (3HB) for short]. As typical examples other than P (3HB), the copolymer [P.A.Holmes et al (ICI). Eur.Pat.Appl. 0052459 (1981)] of 3HB and D-3-hydroxyvalerate (3HV) and the copolymer (Y.Doi et al., Macromolecules, and [21, 2722] (1988)) of 3HB and 4-hydroxy butyrate (4HB) are mentioned. It is known that the biotechnology polyester accumulated in intracellular exists as minute granulation.
[0007] The higher one of the intracellular biotechnology polyester content (henceforth polymer content) processed is desirable, Generally, 20 % of the weight or more has good polymer content as a dried cell. When an alkali addition, the processing time, the effectiveness of separation actuation, the purity of a separation polymer, etc. are taken into consideration, 50% of the weight or more of especially polymer content is desirable. Aqueous suspension points out the thing which made water suspend the biomass separated from the culture suspension after culture termination itself, or culture medium by centrifugal etc. 150 or less g/l of suspension concentration of a biomass is 100 or less g/l preferably in dried cell conversion. As alkali to be used, the hydroxide or NH4 OH of alkali metal including LiOH(s) including NaOH, KOH, etc. is used, the amount of the alkali used -- 1 mmol/kg biomass -1mol a /kg biomass -- desirable -- a 2.5 mmol/kg biomass - 200 mmol/kg biomass -- especially, it is a 50 mmol/kg biomass - 200 mmol/kg biomass preferably, and this is added to the aqueous suspension of a microorganism.
[0008] By the approach of this invention, after adding alkali, aqueous suspension is introduced into the pressure-resistant container which has minute opening, and can apply high voltage. Thus, in order that big shearing force may work in the biomass extruded from opening, a biomass is destroyed and it is presumed that separation of biotechnology polyester is promoted. The equipment which consists of such a pressure-resistant container and an application-of-pressure device is represented by the high voltage homogenizer with a circulation system. Therefore, operation of the biotechnology polyester separation method of this invention is attained by

utilization of a high voltage homogenizer. 40-100 degrees C of temperature setting out of a high voltage homogenizer are preferably made into 60-100 degrees C. As for heating of this suspension, it is also desirable to heat to laying temperature before installation of a high voltage homogenizer. The pressure put on this suspension introduced in the high voltage homogenizer is 500 - 1500 kgf/cm2, although based on equipment. It is desirable to make it act.
[0009] As a high voltage homogenizer with a circulation system, MANTON gaulin (German country APV and gaulin company make), a mini-laboratory (made in Denmark APV Lanny), bulan RYUBE continuous system cell homogenizer (product made from German country Bran+Luebbe), a Micro fluidizer (product made from U.S. Mjcrofluidics), etc. can be used. It is known well that these equipments will be used for emulsification, distribution, cell crushing, etc. by generally pressurizing a liquid. Since heating within a high voltage homogenizer is Mandatory in this invention, the French press which is a non-heating mold although it is a kind of a similar high voltage homogenizer is unsuitable to this invention. Although separating the biotechnology polyester in a microorganism using an French press is known (Helmut Brandl etal., Advances in Biochemical Engineering. Biotechnology (1990), 41, 77-93.), the example which realized common effectiveness of the alkali addition which is the technical feature of this invention, and separation by heating is not known.
[0010] By the above processing actuation, a biomass wall is destroyed efficiently for a short time, and biotechnology polyester can be separated from a biomass by granularity. If a biomass wall is destroyed, since a water-soluble polymeric material like a nucleic acid is eluted out of a cell, the viscosity of this suspension will once rise, but probably because cutting of a nucleic-acid molecule also takes place according to shearing force, the viscosity of this suspension falls again and can separate the biotechnology polyester in subsequent centrifugal actuation, filtration actuation, etc. easily. Since it can process to 150g biomass /l, the cell mass concentration of this suspension before processing does not usually need to thin the cell mass concentration after culture with dried cell conversion. A biomass wall is efficiently destroyed by this invention for a short time, and biotechnology polyester can be seperated by granularity.
[0011]
[Example] microorganism Alcaligenes RIPORITIKA (Alcaligenes lipolytica) AK201 (JP,5-64592,A) to which the microorganism used by this example belongs to Alcaligenes -- it is -- after culture and P (3HB) -- about 50 wt(s)% -- by centrifugal (8000rpm, 10min, and a centrifugal separator are 6810 made from KUBOTA activities), water was added after separation and to a paste-like biomass from culture medium, and the contained bacillus was used as the aqueous suspension of 40g biomass / l. The examples 1 and 2 and the examples 1-4 of a comparison which are shown below were performed using this aqueous suspension.
[0012] P (3HB) obtained by actuation of examples 1 and 2 and the examples 1-4 of a comparison analyzed by using gel permeation chromatography (GPC) for the decision of a gas chromatography and molecular weight distribution in order to investigate purity. In addition, after drying the settlings obtained in examples 1 and 2 and the examples 1-4 of a comparison (105 degrees C, 24hr), what carried out the methanolysis with the methanol/sulfuric acid (85/15 wt%/wt%), and made the polyester in a biomass the methyl ester of a monomer was analyzed to the gas chromatography, and it was asked for polymer content. This followed the approach shown in [H.Brandl et al, Int.J.Biol.Macromol., 11, 49-55 (1989)]. In GPC, after an extract and a solution were condensed for the polyester in a sample (about 100mg) by heat chloroform 150ml, the hexane was added and reprecipitated, it filtered, and the vacuum drying (2hr) of the precipitation was carried out, it was used as the chloroform solution (10mg / 10ml), and it measured.
[0013] (Example 1) The NaOH water solution of 0.1M was added so that it might be set to 4.0mM, and 500ml of these suspension of P (3HB) content biomass was created. This suspension is beforehand thrown into APV and the man ton gaulin by the gaulin company after heating for

shearing force was applied. This actuation was repeated 5 times by circulating suspension automatically. Centrifugal separation (2700rpm, 10min) of the suspension after processing was carried out, and settlings were obtained.
(Example 2) It was operated like the example 1 except making whenever [in MANTON gaulin / carrying-out-for about 5 minutes-preheating of 70 degrees C of these suspension, and stoving temperature] into 70 degrees C.
[0014] (Example 1 of a comparison) In this example, it was operated like the example 1 except not adding a NaOH water solution to this suspension.
(Example 2 of a comparison) In this example, it was operated like the example 2 except not adding a NaOH water solution to this suspension.
(Example 3 of a comparison) In this example, it was operated like the example 1 except not heating this suspension.
The separation conditions of examples 1 and 2 and the examples 1-4 of a comparison are shown in a table 1.
[0015]
[A table 1]

	アルカリ量	予備加熱温度	マントンゴーリンの使用
実施例1	4.0mM	90℃ (5min)	有 (5回)
実施例2	4.0mM	70℃ (5min)	有 (5回)
比較例1	無	90℃ (5min)	有 (5回)
比較例2	無	70℃ (5min)	有 (5回)
比較例3	4.0mM	室温	有 (5回)

The result obtained by the gas chromatography of an example and the example of a comparison and GPC was shown in a table 2.
[0016]
[A table 2]

	ポリマー純度	Mn	Mw	Mw/Mn
実施例1	77.0%	$1.20*10^5$	$3.48*10^5$	2.91
実施例2	85.4%	$3.14*10^5$	$4.86*10^5$	1.55
比較例1	63.4%	$2.10*10^5$	$4.15*10^5$	1.98
比較例2	59.0%	$2.04*10^5$	$3.84*10^5$	1.88
比較例3	65.3%	$2.63*10^5$	$4.35*10^5$	1.66

[0017]
[Effect of the Invention] By this invention, the new separation approach which conquered the fault of the conventional all directions method was developed. That is, biotechnology polyester was separable from the microorganism containing biotechnology polyester by adding little alkali in an aquosity medium, operating a high voltage homogenizer under heating of less than 100 degrees C, and applying shearing force to a biomass without using an organic solvent.